Pharmacology & Toxicology 1992, 70, 322–330.

Studies on the Mechanism of Acetonitrile Toxicity I: Whole Body Autoradiographic Distribution and Macromolecular Interaction of 2-^{14}C-Acetonitrile in Mice*

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(Received May 22, 1991; Accepted October 22, 1991)

Abstract: Acetonitrile, a commonly used solvent is known to cause central nervous system dysfunctions. In order to gain an insight onto the mechanism of acetonitrile toxicity, we studied the kinetics of acetonitrile distribution in mice. Male ICR mice were given a tracer dose of 2-^{14}C-acetonitrile intravenously (60 μ mol/kg or 684 μCi/kg, spec. act. 11.4 mCi/mmol). At various time intervals (5 min., 0.5, 1, 4, 8, 24 and 48 hr) after treatment, mice were anaesthetized and frozen by immersion in a dry ice/hexane mixture, or they were dissected for collection of organs and tissues. Frozen mice were processed for whole body autoradiography, which allows the detection of non-volatile metabolites of acetonitrile at their sites of accumulation. Covalent binding of acetonitrile metabolites in tissues was determined using trichloroacetic acid followed by ethanol/ether extraction techniques. Whole body autoradiography revealed heavy localization of acetonitrile metabolites in the gastrointestinal tissues and bile. At 5 min., the highest levels of radioactivity occurred in the liver and kidney; levels declined over time. At 24 and 48 hr, acetonitrile derived radioactivity were detected in the gastrointestine, thymus, liver and male reproductive organs. Covalent binding studies at 24 and 48 hr after treatment indicated that 40–50% of the total radioactivity present in the liver was bound to the macromolecular fractions of the tissues. The radioactivity contents of other organs were, in large part (40–50% of total), present in the lipid fraction of the tissue. Our studies suggest that the irreversible association with tissues of radioactivity derived from 2-^{14}C-acetonitrile is due to the metabolic activation of acetonitrile and the covalent interaction of reactive metabolite(s) with lipid and macromolecular fractions of the cell.

Acetonitrile is a solvent widely used in polymerization industries and in research laboratories (Ingwalson 1967; Smith 1980). In humans, accidental poisoning and death have resulted from exposure to acetonitrile in various occupational conditions (Amdur 1959; Dequidt *et al.* 1974). In animals, treatment with acetonitrile induces significant increase in teratogenic effects in pregnant hamsters and produces embryotoxicity in rats (Willhite 1983; Zimmerman *et al.* 1985). Systemic toxicity of acetonitrile results in pulmonary and cerebral hemorrhage and thyroid hyperplasia (Pozzani *et al.* 1959; Haguenor *et al.* 1975a & b).

Acetonitrile is known to be metabolized *in vivo* to cyanide, which is excreted as thiocyanate (Lang 1894; Freeman & Hays 1985). *In vitro* studies indicate that acetonitrile metabolism is catalyzed by mixed function oxidase (Tanni & Hashimoto 1984a & b; Freeman & Hays 1988). The proposed metabolic pathway, shown in fig. 1, involves the microsomal oxidation of the alpha carbon atom of acetonitrile and the formation of a reactive intermediate that may be a methylene cyanohydrin. The methylene cyanohydrin possibly undergoes further decomposition to cyanide ions and formaldehyde or undergoes covalent interactions with biological molecules.

In spite of acetonitrile's occupational and environmental importance, little is known about its biologic fate in human or animal models. To obtain further insight into the pharmacokinetics of acetonitrile and its role in acetonitrile toxicity, a clear understanding of its disposition and subcellular interactions is necessary.

The objective of this study is to elucidate the fate of acetonitrile in biological systems by using mice as an animal model. We have studied: A) the distribution of 2-^{14}C-acetonitrile and its metabolites in mice, using whole body autoradiography and classical dissection techniques, and B) the accumulation and chemical interaction of acetonitrile and/or its metabolites in various tissue fractions.

Materials and Methods

Chemicals. 2-^{14}C-Acetonitrile (spec. act. 11.4 mCi/mmol, 98% purity) was obtained from Amersham (Arlington, IL, U.S.A.). Chemical and radiochemical purity of 2-^{14}C-Acetonitrile was established using gas-liquid chromatography techniques (column 10% carbowax 1540 on Chromosorb (waw), temperature 50°, nitrogen flow rate 1 ml/min.). Acetonitrile (HPLC grade) was obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). Carbo-Sorb II and Permafluor V were obtained from Packard Instrument Company (Laguna Hills, CA, U.S.A.). Aquasol-2 was purchased from New England Nuclear Research Products (Boston, MA, U.S.A.). The specific activity of the dosing solution was determined in PCS scintillation fluor (Amersham), using a Searle Mark III, model 6880 (Searle Analytic, Inc., Chicago, IL, U.S.A.) liquid scintillation counter, and counting efficiency was determined by external standardization. All

* Preliminary results were presented at the 27th Annual Meeting of the Society of Toxicology, February, 1988, Dallas, TX, U.S.A.

other chemicals used were the highest purity commercially available. All experiments were conducted in well-ventilated hoods.

Animal care and treatment. Male ICR mice (Sprague Dawley, Houston, TX) weighing 30–35 g were used. The animals were acclimatized in our animal facility, for one week prior to treatment, with free access to Purina chow and drinking water. Each mouse received (intravenously into the tail vein) a tracer dose of 2-^{14}C-Acetonitrile (684 μCi/kg equivalent to 60 μmol/kg). Immediately following treatment, the animals were placed, three per group, in all-glass metabolism cages (Vanguard International, Inc., Neptune, NJ, U.S.A.) with free access to food and water. Animals in each group were taken for freezing or dissecting at specified time intervals, i.e. 5 min. and 0.5, 1, 4, 8, 24, and 48 hr, following treatment as described below.

Tissue distribution of 2-^{14}C-acetonitrile metabolites using whole body autoradiography. At specified time intervals, animals were killed by CO_2 inhalation, embedded in carboxymethyl cellulose molds, and frozen in dry ice/n-hexane cooled to −70°. Frozen animals were then stored at −20° until the time of sectioning. Briefly, frozen animals were sectioned using LKB PMV 2250 cryomicrotome (Stockholm, Sweden) at −17°. Sections were taken in sagittal plane, at thickness of 20 and 60 μm, on Scotch tape 810 (3M Co., Minneapolis, MN, U.S.A.). The sections were then freeze-dried at −20° in the cryostat. Autoradiography was performed by affixing the freeze-dried sections to Industrex AA X-ray film (Kodak Industries), with exposure period up to 4 weeks at −20°. Completed autoradiographs were developed and printed by standard photographic procedures. Representative autoradiographs were selected and used as negatives in a photographic enlarger to produce the prints shown in fig. 2–4. Therefore, in the autoradiograph figures white areas represent sites of radioactivity uptake and the grayness level is inversely proportional to the radioactivity.

The whole body autoradiographic procedure demonstrates the sites of inter- and intraorgan localization of nonvolatile acetonitrile metabolites as they existed *in vivo* at the time of freezing. This approach minimizes kinetic disturbances that occur as a result of blood loss during normal dissection techniques.

Tissue distribution of 2-^{14}C-acetonitrile and its metabolites using standard dissection technique. Upon sacrifice, the animals were lightly anaesthetized with ether, an abdominal incision was made, and blood samples were collected from the portal vein into heparinized tubes. The total radioactivity in whole blood was determined. After blood withdrawal, various tissues were excised, rinsed in cold saline, blotted dry with filter paper, weighed and minced. The total radioactivity (acetonitrile and metabolites) in cold, preweighed tissue samples was determined using a biological sample oxidizer (model B306, Packard Instrument Co., Donners Grove, IL, U.S.A.) and scintillation counter. To determine tissue contents of nonvolatile radioactive metabolites, aliquots from tissues were heated on a hotplate at 80° for one hour prior to processing in the sample oxidizer. The difference between total tissue radioactivity and that of the nonvolatile radioactivity represent 2-^{14}C-acetonitrile concentration in each tissue. The radioactivity contents of each sample was converted to μg-equivalent of acetonitrile, based on the specific activity of the dose administered.

Kinetic parameters and apparent half-lives of 2-^{14}C-acetonitrile and non-volatile metabolites in blood and each tissue were determined using the computer program, NONLIN (Statistical Consultants, Inc., Lexington, KY, 1984).

Covalent interactions of acetonitrile metabolites with tissue macromolecules. To determine the covalently bound radioactivity in the macromolecular fractions of the cells, tissues were homogenized in 1.15% KCl solution. Known aliquots of homogenates of various tissues were treated overnight with cold, 10% (w/v), trichloroacetic acid. After centrifugation, the precipitates were washed twice with 5% trichloroacetic acid, twice with ethanol/ether (1:3, v/v) to remove lipid fraction, and finally with 70% ethanol to insure the complete removal of any reversibly bound radioactivity in the macromolecular fraction (proteins and nucleic acids). To determine the radioactivity bound to proteins and nucleic acids, the final dried precipitates were dissolved in 0.1N NaOH, and radioactivity was counted after mixing with Aquasol-2. Based on the specific activity of the dosing solution, the radioactivity content of each chemical fraction was converted to μg-equivalent acetonitrile.

Subcellular distribution of acetonitrile metabolites in the liver. Weighed samples of the liver were minced and homogenized in 10 volumes of 0.25 M sucrose, using a motor driven teflon Potter-Elvehjem homogenizer. Subcellular fractionation of tissue homogenates was carried out by the standard differential centrifugation method (Ahmed *et al.* 1983). The purity of individual subcellular fraction was determined by using standard markers as described before (deDuve *et al.* 1955; deDuve 1971). Total radioactivity in tissue homogenates and subcellular fractions was assessed with a biological sample oxidizer and scintillation counter. Based on the specific activity of the administered dose, the radioactivity content of each fraction was converted to μg-equivalent acetonitrile.

Statistical analysis. GraphPad (ISI Software, Philadelphia, PA, U.S.A.) computer programs were used in regression analysis of collected data. Data were analyzed by Student's t-test and expressed as means ± S.D.

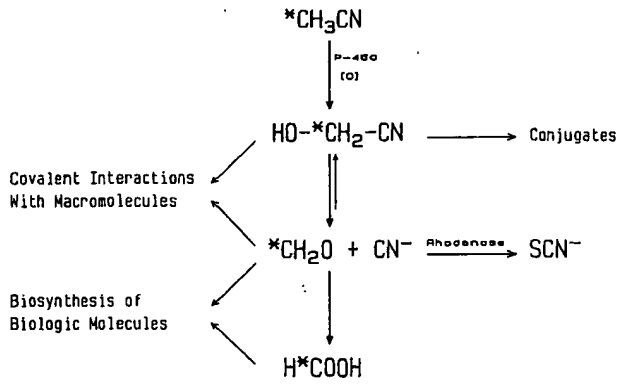

Fig. 1. Proposed metabolic pathway for acetonitrile.

Results

Whole body autoradiographic distribution of 2-^{14}C-acetonitrile non-volatile metabolites.

The autoradiograms of mice treated with 2-^{14}C-acetonitrile are shown in fig. 2–4. At 5 min. after administration of 2-^{14}C-acetonitrile, the highest concentrations of non-volatile radioactive metabolites were observed in the liver, kidney, and the contents of the upper gastrointestinal tract, gall bladder, and urinary bladder (fig. 2). High radioactivity was also observed in the nasal secretions as well as the lining of the mouth cavity. The autoradiogram in fig. 2 indicates that skin and cartilage surrounding various bone structures contained an extensive amount of radioactivity. Radioactivity in blood at this time was high; however, it was lower than that in the liver. Even though blood contained substantial amounts of radioactivity, the ^{14}C contents of myocardial tissues were minimal. The accumulation of radioactivity

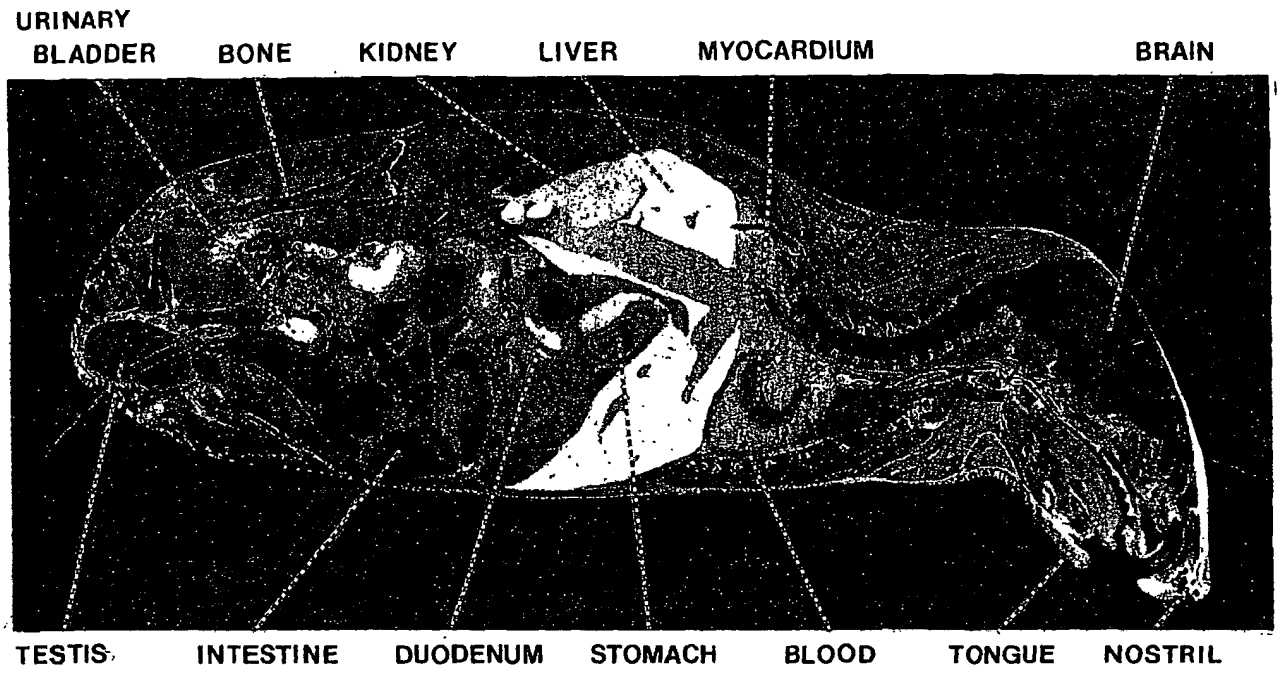

Fig. 2. Whole body autoradiogram of a freeze-dried sagittal section of a mouse killed 5 min. after intravenous injection of 2-^{14}C-acetonitrile (2.46 mg/kg). White areas correspond to high levels of radioactivity. Note excessive accumulation in liver, kidney, and the contents of gall bladder, urinary bladder and upper intestine.

in salivary glands, the tongue, the esophagus and other gastrointestinal tissues was not high. The brain contained the least amount of non-volatile radioactivity at 5 min. following 2-^{14}C-acetonitrile administration. Although no accumulation of radioactivity was observed in the testes at this point (5 min.), the epididymis and the penal tissues contained a detectable level of nonvolatile ^{14}C.

At 1, 4 and 8 hr following treatment of 2-^{14}C-acetonitrile,

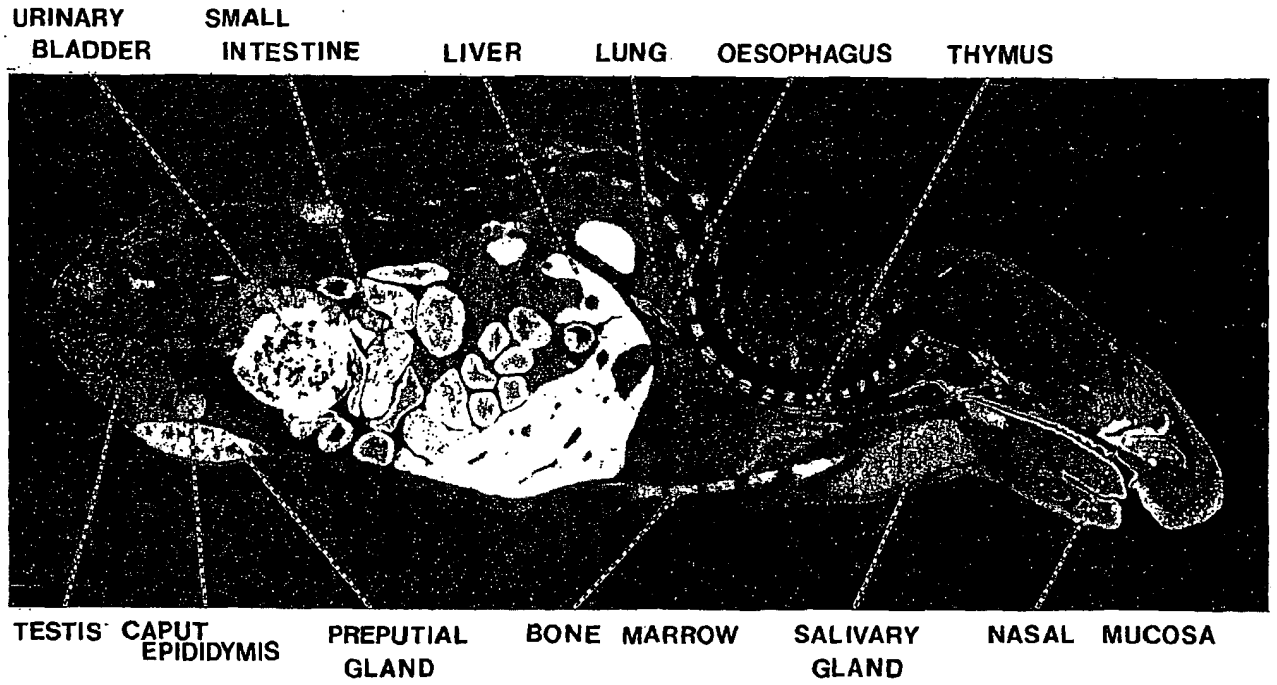

Fig. 3. Whole body autoradiogram of a freeze-dried sagittal section of a mouse killed 24 hr after intravenous injection of 2-^{14}C-acetonitrile (2.46 mg/kg). White areas correspond to high levels of radioactivity. Note excessive accumulation in liver, gastrointestinal walls, bone and cartilage, prostate gland, and prepuatil gland.

a very high concentration of non-volatile radioactive acetonitrile metabolites were detected in the contents of urinary bladder, gall bladder, stomach, and intestinal tract. Tissues of other structures such as esophagus, salivary glands, liver, gastrointestinal tract and kidney contained moderate levels of non-volatile radioactivity (data not shown).

The distribution of non-volatile radioactivity at 24 hr following the administration of 2-^{14}C-acetonitrile was also studied (fig. 3). At end of this time period, a more differentiated pattern of distribution into peripheral compartments was observed. While the levels of radioactivity sharply declined in circulating fluids and contents, its accumulation in specific tissues was observed. High levels of ^{14}C were retained in the liver, kidney and urinary bladder, and in the contents of the large intestinal tract, such as in cecum. At this time high levels of non-volatile ^{14}C were observed in bone marrow of the spine and sternum. Accumulation of non-volatile radioactivity was also observed in the outer lining of the tongue, and in the upper gastrointestinal tissues, such as oesophagus, stomach walls and upper intestinal tract. The salivary and the thymus glands contained a levels of radioactivity that was higher than levels observed in blood (fig. 3). The male reproductive organs, such as the testes and the prostate gland, showed gradual accumulation of ^{14}C, while the preputial gland contained radioactivity comparable to that present in the liver (fig. 3). At 24 hr following treatment, the brain contained the least amount of non-volatile radioactivity.

The delayed retention and incorporation of radioactivity in mouse tissues following the administration of 2-^{14}C-acetonitrile was also studied (fig. 4). At 48 hr post-treatment, most of the circulating radioactivity was excreted. The remaining radioactivity was firmly bound or incorporated in the macromolecular fractions of tissues. The liver and gastrointestinal mucosa, submucosa and muscularis contained the highest levels of ^{14}C. The lining of the mouth cavity and the tongue also contained considerable amounts of radioactivity. Bone marrow, submaxillary salivary gland, thyroid gland, thymus gland, as well as the nasal mucosa contained radioactivity that was higher than that present in the heart and blood. Of interest is the retention of labeled metabolites in the male reproductive organs such as the testes, prostate and preputial glands. Delayed accumulation and retention of 2-^{14}C-acetonitrile metabolites was also observed in the brain (fig. 4) at the end of this time period.

Quantitative determination of 2-^{14}C-acetonitrile and its metabolites in mouse tissues.

The levels of total radioactivity (acetonitrile and metabolites) as a function of time in μg-equivalent acetonitrile/g tissue are shown in table 1. Following a single i.v. dose of 2-^{14}C-acetonitrile, the highest levels of radioactivity in all tissues were found at the earliest time interval (5 min.) after treatment. Both kidney and bladder contained high levels of radioactivity. While the radioactivity in the kidney declined with time, increased radioactivity in bladder contents was observed. This shift indicates that 2-^{14}C-acetonitrile and its metabolites were rapidly excreted from blood to kidney, to urinary bladder, and to the urine. Similarly, high levels of radioactivity were observed in the liver, spleen, testes and

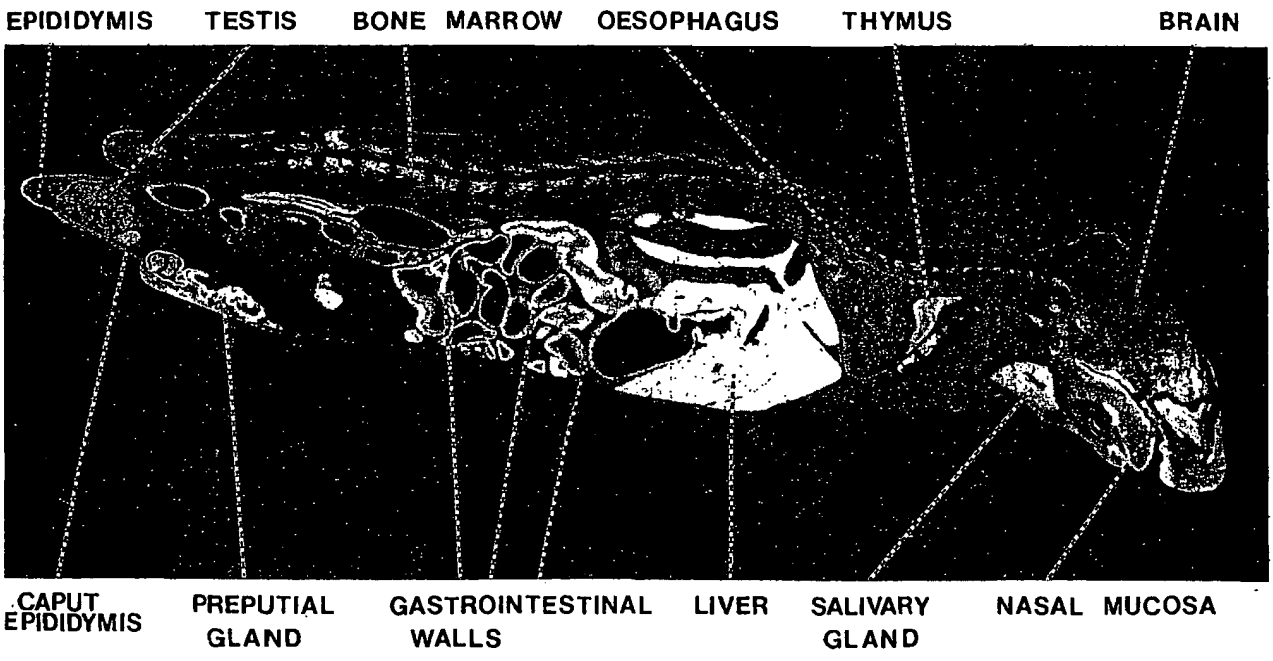

Fig. 4. Whole body autoradiogram of a freeze-dried sagittal section of a mouse killed 48 hr after intravenous injection of 2-^{14}C-acetonitrile (2.46 mg/kg). White areas correspond to high levels of radioactivity. Note delayed accumulation in liver, gastrointestinal walls, thymus, nasal cavities, and reproductive organs.

Table 1.

Tissue levels of total radioactivity at various time intervals following intravenous administration of 2-^{14}C-acetonitrile (ACN) (2.46 mg/kg).

Tissue	μg eq. 2-^{14}C-ACN/g tissue*						
	5 min.	0.5 hr	1 hr	4 hr	12 hr	24 hr	48 hr
Eyes	0.43	0.50	0.33	0.41	0.13	0.07	0.04
Blood	0.78	1.16	0.83	0.88	0.34	0.98	0.58
Brain	0.57	0.45	0.45	0.44	0.16	0.05	0.04
Heart	0.94	0.65	0.61	0.50	0.27	0.15	0.10
Lung	0.83	0.61	0.48	0.51	0.18	0.21	0.16
Thymus	0.54	0.40	0.29	0.28	0.23	0.21	0.15
Liver	2.11	1.26	1.03	0.96	1.27	1.03	0.39
Spleen	0.97	0.80	0.77	0.59	0.55	0.41	0.17
Pancreas	0.72	0.62	0.63	0.59	0.43	0.18	0.11
Stomach	0.82	0.59	0.52	0.37	0.45	0.31	0.14
Small intestine	2.22	1.40	0.74	0.35	0.36	0.65	0.32
Large intestine	0.35	0.31	1.71	5.04	2.08	0.75	0.12
Duodenum	0.79	1.14	0.49	0.66	0.56	0.38	0.43
Kidney	1.78	1.02	0.91	0.67	0.65	0.54	0.32
Adrenal	0.54	0.43	0.26	0.23	0.10	0.28	0.16
Bladder	2.17	4.08	3.46	2.54	1.34	0.45	0.22
Epididymis	0.44	0.69	0.26	0.29	0.17	0.20	0.19
Testes	1.12	1.32	1.08	1.04	0.42	0.18	0.17
Skin	1.03	0.63	0.60	0.54	0.26	0.18	0.10
Bone	0.31	0.22	0.25	0.24	0.12	0.08	0.04
Fat	0.11	0.14	0.06	0.36	0.06	0.08	0.08

* Values are means of two animals.

Table 2.

Distribution coefficients of 2-^{14}C-acetonitrile equivalents following a single intravenous dose of 2-^{14}C-acetonitrile (2.46 mg/kg).

Tissue	Time			
	5 min.	1 hr	12 hr	24 hr
Eyes	0.55	0.40	0.38	0.07
Brain	0.73	0.54	0.47	0.05
Heart	1.21	0.73	0.79	0.15
Lung	1.06	0.58	0.53	0.21
Thymus	0.69	0.35	0.68	0.21
Liver	2.71	1.24	3.74	1.05
Spleen	1.24	0.93	1.62	0.42
Pancreas	0.92	0.76	1.26	0.18
Stomach	1.05	0.63	1.32	0.32
Small intestine	2.85	0.89	1.06	0.66
Large intestine	0.45	2.06	6.12	0.77
Duodenum	1.01	0.59	1.65	0.39
Kidney	2.28	1.10	1.91	0.55
Adrenal	0.69	0.31	0.29	0.29
Bladder	2.78	4.17	3.94	0.46
Epididymis	0.56	0.31	0.50	0.20
Testes	1.44	1.30	1.24	0.18
Skin	1.32	0.72	0.76	0.18
Bone	0.40	0.30	0.35	0.08
Fat	0.14	0.07	0.18	0.08

Distribution coefficient is the ratio of μg equivalent acetonitrile per g tissue to μg equivalent acetonitrile per ml blood.

skin. The total radioactivity in the liver was found to be higher than that in blood at all times except at 48 hr. Significantly higher levels in these tissues than those observed in blood indicates a fast transport rate from blood to tissues. High levels of acetonitrile metabolites were also observed in small intestinal tissues. The radioactivity in the gastrointestinal tract had moved from the small intestine to the large intestine and it was highest in the large intestine at 4 hr following treatment. As with acrylonitrile (Ahmed *et al.* 1982), the spleen was a target for the accumulation and retention of radioactivity following acetonitrile administration. Detectable amounts of radioactivity were still present in all tissues and blood at 48 hr after treatment.

Distribution coefficients assess the transport of 2-^{14}C-acetonitrile and its metabolites between blood (central compartment) and various tissues (peripheral compartments). The distribution coefficients of 2-^{14}C-acetonitrile equivalents (ratio of μg-equivalent of 2-^{14}C-acetonitrile in g tissue to that in ml blood) in major tissues were determined (table 2). The data in table 2 indicate that although 2-^{14}C-acetonitrile was administered intravenously, most tissues had radioactivity equal to or higher than that in blood at 5 min. after treatment. The excessive transport of 2-^{14}C-acetonitrile from blood to tissues is indicated by high distribution coefficients in the liver, kidney, small intestine, bladder, testes, spleen, and skin. In most tissues the coefficients were higher than one before the first hour after treatment and declined gradually over the period of observation.

Pharmacokinetic parameters of total 2-^{14}C-acetonitrile equivalents in several tissues are shown in table 3. Elimination rate constants varied between tissues. The apparent fast elimination rates ($t_{1/2}\alpha$) were in the range of 0.08 hr in the skin to 1.77 hr in the eyes. The apparent slow elimination rates ($t_{1/2}\beta$) were between 8.60 hr in the urinary bladder to 536.26 hr in the small intestinal tissues. The area under the curve, as a function of time, was found to be extremely high in the small intestine (269 μg/g hr) and moderate in liver and adrenal glands (85 and 66 μg/g hr, respectively).

Table 3.

Areas under the curves and apparent half lives of total radioactivity in various tissues following intravenous administration of 2-^{14}C-acetonitrile (2.46 mg/kg).

Tissue	$t_{1/2}\alpha$ (hr)	$t_{1/2}\beta$ (hr)	AUC (μg/g·hr)
Thymus	0.32	54.26	21.99
Liver	0.12	50.37	85.48
Kidney	0.22	38.59	49.54
Spleen	0.43	27.45	27.86
Heart	0.14	11.48	10.55
Lung	0.27	18.87	13.38
Stomach	0.30	34.49	23.77
Skin	0.08	12.26	11.36
Bladder	0.30	8.60	44.26
Small intestine	0.45	536.26	268.65
Epididymis	0.10	89.21	34.40
Eyes	1.77	19.80	5.99
Adrenal	0.58	272.70	66.20

AUC: Area under the curve.

$t_{1/2}\alpha$, $t_{1/2}\beta$: Apparent rapid and slow elimination half-lives of radioactivity.

Distribution of 2-^{14}C-acetonitrile in tissues.

The levels of 2-^{14}C-acetonitrile in mouse tissues as a function of time in units of μg-equivalents of 2-^{14}C-acetonitrile/g tissue, are shown in table 4. Following a single intravenous dose of 2-^{14}C-acetonitrile, the highest levels of acetonitrile in most tissues were observed at 5 min. following treatment. Tissues with the highest levels of 2-^{14}C-acetonitrile (0.9 μg-equivalent/g tissues) were the liver and testes. At 0.5, 1, and 4 hr following treatment, the concentration of 2-^{14}C-acetonitrile in the testes were slightly equal to or higher than that in blood. Other organs contained variable levels of 2-^{14}C-acetonitrile concentration at various time intervals. Of interest is the considerable amount of 2-^{14}C-acetonitrile in the brain. The levels of acetonitrile in the brain was almost equal to or slightly higher than that in the blood at 5 min. after treatment. No 2-^{14}C-acetonitrile was detectable in brain tissues after 24 hr following treatment.

The data in table 4 were used for curve fitting pharmacokinetic analysis of 2-^{14}C-acetonitrile in mouse blood and tissues using PC NONLIN pharmacokinetic programs. Blood or tissue levels of 2-^{14}C-acetonitrile were best fit in one compartment model with bolus input and first order output (table 5). The area under the curve for acetonitrile ranged from 4.20 μg/g hr for the lung to 10.75 μg/g hr in the testes. The half-life of elimination of 2-^{14}C-acetonitrile from blood and most tissues ranged from 5.52 hr in the liver to 8.45 hr in the blood. The liver, which is the major site for acetonitrile metabolism, has the shortest elimination half-life of 2-^{14}C-acetonitrile intact molecule.

Covalent binding of 2-^{14}C-acetonitrile metabolites in various tissues.

The homogenates of several tissues were fractionated into acid soluble, lipid, and macromolecular fractions. The contents of ^{14}C of each fraction as a function of time were determined. The association of ^{14}C in the lipid and macromolecular fractions in each tissue is shown in table 6. The data in table 6 shows the time dependence of alkylation of lipids and macromolecular fractions in terms of μg-equivalent of 2-^{14}C-acetonitrile per g tissue. In all tissues examined about 5–10% of the total ^{14}C from 2-^{14}C-acetonitrile was covalently bound to macromolecular fraction in the first hour. The highest covalent binding was observed in the lung and pancreas (10–11% of total). Irreversible interaction of radioactivity with lipid fraction of the cell was about 6–16% of total at 1 hr following treatment. Although the total radioactivity in tissues declined over time, the relative percentage of radioactivity covalently bound to lipids, proteins, and nucleic acids increased (table 6). By 48 hr following treatment with 2-^{14}C-acetonitrile about 25–45% of the radioactivity present in the tissues was covalently bound to macromolecules, and 30–50% was bound to lipids. Both hepatic and pancreatic tissues contained the highest macromolecular binding of radioactivity at 24 and 48 hr after treatment.

Table 4.

Tissue levels of acetonitrile (ACN) following a single intravenous dose of 2-^{14}C-acetonitrile (2.46 mg/kg).

	μg eq. 2-^{14}C-acetonitrile/g tissue*					
Tissue	5 min.	0.5 hr	1 hr	4 hr	12 hr	24 hr
Blood**	0.42	0.97	0.72	0.83	0.23	0.00
Liver	0.92	0.68	0.63	0.54	0.18	0.02
Kidney	0.59	0.51	0.55	0.41	0.16	0.00
Testes	0.89	0.92	0.90	0.91	0.25	0.00
Spleen	0.67	0.59	0.62	0.42	0.22	0.02
Heart	0.64	0.47	0.49	0.40	0.14	0.00
Lung	0.40	0.37	0.31	0.38	0.08	0.01
Pancreas	0.29	0.42	0.42	0.37	0.10	0.00
Brain	0.50	0.39	0.40	0.40	0.11	0.00

* Values are means of two animals.
** μg equivalent 2-^{14}C-ACN/ml blood.

Table 5.

Apparent half-lives of acetonitrile in various tissues of mice treated with intravenous dose of 2-^{14}C-acetonitrile (2.46 mg/kg).

Tissue	AUC* (μg/g · hr)	K_{10}-HL** (hr)
Blood	10.00	8.45
Brain	4.61	6.77
Heart	4.90	5.88
Lung	4.20	7.28
Liver	6.44	5.52
Spleen	6.36	6.68
Kidney	5.41	6.38
Testes	10.75	7.53
Pancreas	4.52	7.69

* AUC: Area under the curve.
** K_{10}-HL: Apparent elimination half-life of acetonitrile.

Distribution of radioactivity in hepatic subcellular fractions.

The subcellular distribution and the incorporation of radioactivity in hepatic tissues were studied as a function of time (table 7). The data in table 7 show the μg-equivalent of 2-^{14}C-acetonitrile in each subcellular fraction.

At 5 min. following treatment the radioactivity derived from 2-^{14}C-acetonitrile was indiscriminately distributed in all subcellular fraction; the radioactivity in each fraction was a function of time. The data in table 7 indicate that the cytosolic fraction contained the highest level of radioactivity at all time periods. Mitochondrial and nuclear membrane fractions contained similar levels of ^{14}C, while the microsomal fraction contained the least amount. Further chemicals fractionation studies indicated that at early time intervals (0–4 hr) following 2-^{14}C-acetonitrile administration, the majority of the radioactivity present in the cytosolic, nuclear membrane, mitochondrial, and microsomal fractions correspond to metabolized acetonitrile. At 24 hr after treatment all radioactivity present in hepatic fractions were found in the form of metabolites. This observation suggests that fractions of the hepatic cells other than microsomal maybe involved in the metabolic biotransformation of acetonitrile.

Table 6.

Covalent binding of 2-^{14}C-acetonitrile metabolites to lipids and macromolecular fractions (proteins and nucleic acids) of various tissues homogenates following a single intravenous dose of 2-^{14}C-acetonitrile (2.46 mg/kg).

	µg eq. 2-^{14}C-ACN/g tissue								
	1 hr			24 hr			48 hr		
Tissue	Total	Lipid	MM*	Total	Lipid	MM*	Total	Lipid	MM*
Liver	1.03	0.11 (11%)†	0.04 (4%)	1.03	0.29 (28%)	0.48 (47%)	0.39	0.13 (33%)	0.16 (42%)
Kidney	0.91	0.14 (15%)	0.05 (5%)	0.54	0.23 (43%)	0.19 (35%)	0.32	0.16 (50%)	0.11 (33%)
Heart	0.61	0.05 (8%)	0.05 (8%)	0.15	0.05 (34%)	0.06 (40%)	0.10	0.04 (36%)	0.04 (36%)
Lung	0.48	0.08 (16%)	0.05 (10%)	0.21	0.08 (38%)	0.08 (38%)	0.16	0.07 (41%)	0.05 (34%)
Brain	0.45	0.03 (7%)	0.02 (4%)	0.05	0.03 (60%)	0.01 (20%)	0.04	0.02 (50%)	0.01 (25%)
Pancreas	0.63	0.06 (9%)	0.07 (11%)	0.18	0.06 (33%)	0.08 (44%)	0.11	0.05 (45%)	0.05 (45%)
Spleen	0.77	0.06 (8%)	0.05 (6%)	0.41	0.20 (49%)	0.14 (35%)	0.17	0.09 (53%)	0.05 (29%)
Testes	1.08	0.06 (6%)	0.05 (4%)	0.18	0.06 (33%)	0.06 (33%)	0.17	0.06 (35%)	0.85 (29%)

* MM: Macromolecules.

† Numbers in parentheses are percent of total radioactivity.

Discussion

Acetonitrile, a polar solvent and a volatile compound, undergoes biotransformation in various species. Acetonitrile is known to be metabolized to cyanide ion (CN^-) in biological systems (Lang 1894). Cyanide is then detoxified to thiocyanate and excreted in the urine. In general, available information on metabolism of acetonitrile deals only with cyanide formations and suggests the metabolic pathway shown in fig. 1. The proposed pathway shown in fig. 1 suggests that the volatile compound, acetonitrile is converted to non-volatile metabolite, namely formaldehyde cyanohydrin. The latter may undergo further metabolism to release cyanide and formaldehyde, or it may covalently bind to tissue macromolecules via nucleophilic substitution on the electrophilic methylene carbon atom of the metabolite formaldehyde cyanohydrin. Furthermore, formaldehyde may also covalently bind to nucleophilic sites on tissue macromolecules via hydroxymethylene formation (Feeney *et al.* 1975), become incorporated in the *de novo* synthesis of several endogenous compounds, (Ntundulu *et al.* 1976), or undergo further metabolism to formic acid.

Information and knowledge of the metabolic profile of acetonitrile is essential to understand and interpret its distribution in various organs in animal models. In the present study we investigated the distribution of acetonitrile and its metabolites using $^{14}CH_3CN$ molecule. Thus our study did not seek the distribution of the cyanide group, rather, we investigated the distribution of either the whole acetonitrile molecule, or metabolites with or without the cyanide group as shown in fig. 1.

The present study shows that several tissues, in addition to the liver, have a capacity to metabolize and accumulate acetonitrile. Metabolism of acetonitrile is remarkably active in the liver and kidney as indicated by the rapid accumulation of non-volatile acetonitrile metabolites in these organs 5 min. after treatment (fig. 2). However, accumulation of acetonitrile metabolites in the kidney may also employ rapid excretion of urinary metabolites.

High concentrations of ^{14}C in the liver were found

Table 7.

Distribution of total radioactivity in hepatic subcellular fractions following a single intravenous dose of 2-^{14}C-acetonitrile (ACN) (2.46 mg/kg).

	µg eq. 2-^{14}C-ACN/g tissue*					
	5 min.		4 hr		24 hr	
Fraction	Metabolites	ACN	Metabolites	ACN	Metabolites	ACN
Cytosol	0.65	0.47	0.18	0.29	0.37	0
Nuclear membrane	0.11	0.06	0.04	0.04	0.11	0
Mitochondria	0.16	0.04	0.08	0.02	0.21	0
Microsome	0.06	0.02	0.04	0.01	0.07	0

* Values are means of 2 animals.

throughout all survival times tested (fig. 2 and 3). The high level of ^{14}C in the liver indicates a very rapid metabolism of acetonitrile and suggests that hepatic tissues have a dominating role in the metabolism of the compound. When we compared the whole body autoradiography distribution of acetonitrile described in our studies with the distribution of the proposed metabolite, formaldehyde, described by Johansson & Tjälve (1979), we found that the hepatic uptake and retention of radioactivity derived from the two compounds is strikingly different. Much higher radioactivity was detected in the livers of animals treated with 2-^{14}C-acetonitrile as compared to those treated with $^{14}CH_2O$. The accumulation of ^{14}C in the livers of animals treated with 2-^{14}C-acetonitrile, however, was similar to that observed in the livers of animals treated with N,N-dimethyl nitrosamine (Johansson & Tjälve 1979). In their studies, Johansson & Tjälve reported that livers of animals that were treated with dimethylnitrosamine contained much higher levels of ^{14}C compared to the livers of animals that were treated with $^{14}CH_2O$. Therefore, contrary to formaldehyde, the prolonged uptake and retention of the high radioactivity observed in the livers of animals treated with acetonitrile (and N,N-dimethylnitrosamine) may indicate that these latter compounds, may undergo metabolic oxidation reactions and/or other metabolic reactions (such as conjugations) in the liver.

The persistence of radioactivity in the liver suggests a) the formation of reactive electrophilic intermediates that covalently bound to macromolecules of the hepatic cells and/or b) the incorporation of radioactivity, derived from acetonitrile, in the *de novo* synthesis of biological molecules through the one-carbon pool in hepatic tissues. Covalent binding studies indicated the irreversible interaction of radioactivity with macromolecules of most tissues, particularly the liver and gastrointestinal tract. On the other hand, the distribution of radioactivity from 2-^{14}C-acetonitrile in tissues with rapid cellular turnover, such as blood forming organs, the lymphoid system and tissues with high rate of protein synthesis, such as the exocrine pancreas and salivary glands, was similar to the distribution of radioactivity from $^{14}CH_2O$ (Johansson & Tjälve 1979). Therefore, this pattern may be explained on the basis of incorporation of radioactivity via CH_2O intermediate into one-carbon pool of the cell.

Acetonitrile is known to be a neurotoxic agent (Haguenor *et al.* 1975a & b). The whole body autoradiographic study suggests that acetonitrile metabolites are not able to penetrate the blood brain barrier (see above). Quantitative distribution studies of acetonitrile uptake, however, indicate that acetonitrile itself was able to cross the blood brain barrier (table 4). Thus the observed acetonitrile neurotoxicity may be due to the parent acetonitrile molecules and not due to acetonitrile metabolites.

Reproductive and teratogenic effects of acetonitrile have been reported (Johannsen *et al.* 1986). Our studies suggest that acetonitrile and its metabolites cross the blood testes barrier and accumulate in high levels in the tissues of male reproductive organs such as the testes, prostate, and preputial glands. The role of the accumulation of acetonitrile and its metabolities in the male reproductive tissues in acetonitrile-induced reproductive toxicity is not yet known.

Pharmacokinetics analysis of acetonitrile in tissues, indicate that the distribution and excretion kinetics of the acetonitrile parent molecule in various tissues follows one compartment, first order kinetics. Meanwhile, the distribution of acetonitrile metabolites in the tissues follows a two compartment model with elimination half-lives much longer than that for acetonitrile.

In conclusion, our studies indicate that, similar to dimethylnitrosamine, acetonitrile undergoes bioactivation, mostly in the liver, to a reactive metabolite that undergoes irreversible interaction with tissue macromolecules, particularly in the liver and the gastrointestinal tissues, and this reactive intermediate may be transformed to the formaldehyde. Hence, the similarity of uptake, distribution and incorporation of radioactivity derived from 2-^{14}C-acetonitrile to that derived from $^{14}CH_2O$ in tissues of high cellular turn-over.

Acknowledgements

This work is supported by NIH Grant No. ES 01871 awarded by the National Institute of Environmental Health Sciences. We thanks Ms. Maxine Fuller and Ms. Winifred Bourgeois for preparing this manuscript. Also we thank Mr. Sam Jacob for technical assistance. Our deepest appreciation is for Ms. Janice Longoria for editorial assistance of the manuscript.

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